Remarks

This is in response to the Official Action of July 16, 2010. Claims 2-9, 11-31, and 51-59 are pending in the application.

Claims 3, 15, 20, 22-27, 52, and 54-59 have been withdrawn from consideration as directed to nonelected species. Applicant has elected the species where the article is an orthopedic implant, the protein-resistant head group is tri(sarcosine), and the surface portion comprises metal.

Claims 28 and 51 have been amended herein to incorporate the subject matter of claim 17, which has been cancelled.

New claims 60 and 61 have been added herein and recite wherein the contacting step is carried our for a time period of up to one month; claims 62 and 63 have been added herein and recite wherein the contacting step is carried out for a time period of up to approximately 26 days (responsive to the Examiner's comment in the most recent enablement rejection, discussed below). It is believed that no new matter is added by these amendments and new claims. Thus, their entry and consideration are respectfully requested.

Claims 28 and 51 are amended herein to recite a preferred density range: **wherein said brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2.** Support is found in the specification at page 11, lines 20-22, and originally presented claim 17. As discussed in greater detail below, the reference cited by the examiner that specifically immobilizes non-fouling moieties on a surface (**Zhang et al.**) does so at a relatively low density. In that literature, it was believed that such polymers exhibited non-fouling properties partly due to a high degree of chain flexibility/mobility, which results in a relatively large "excluded volume" (i.e., the volume that the chain functionally occupies). **In contrast, in the present invention, reactions are chosen that result in relatively high density polymer immobilization.** If one followed the cited literature, such high density immobilization would NOT be indicated, because it would result in a relatively low "excluded volume" per immobilized chain. The present applicants take this different approach because they have recognized that non-fouling behavior is dependent upon properties beyond simply "excluded volume," such as bound water of hydration. The combination of methods used here was in some aspects counter-intuitive--by following the cited literature, combining these methods to create a high density of immobilized chains bearing non-fouling moieties, with a relatively low excluded volume per

chain, would not have been indicated to result in an enhancement of the non-fouling properties of these materials.

In light of these amendments and the following remarks, Applicant respectfully requests reconsideration of this application and allowance of the pending claims to issue.

I. Claim Objections

Claim 28 is objected to due to a typographical error. Claims 28 has been amended to correct this typographical error. Accordingly, Applicant respectfully submits that this objection is moot and requests its withdrawal.

II. Rejection under 35 U.S.C. § 112, first paragraph – Enablement

Claims 2, 4-9, 11-14, 16-19, 21, 28-31, 51, and 53 stand rejected as allegedly lacking enablement. The Examiner acknowledges that the specification is enabled for methods where the contacting occurs for approximately 26 days or less, but alleges that the specification does not "reasonably provide enablement for the method of use as claimed when the linking layer is an alkanethiol terminated initiator on gold and the contacting occurs for more than 26 days in a biological fluid." Office Action, page 3.

The test for enablement is "whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." *In re Wands*, 858 F.2d, 731, 737, 8 USPQ2d, 1400, 1404 (Fed. Cir. 1988). The key word is "undue," not "experimentation," and "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *Id.*, 8 USPQ2d at 1404 (quoting *In re Jackson*, 217 USPQ 804, 807 (CCPA 1982)).

The factors to be considered in determining whether the experimentation is "undue" include: 1) the nature of the invention, 2) the breadth of the claims, 3) the state of the prior art, 4) the level of one of ordinary skill, 5) the level of predictability in the art, 5) the amount of direction provided by the inventor, 6) the existence of working examples, and 7) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *See, In re Wands, supra.*

Applicant respectfully asserts that, consistent with the conclusions drawn from the *Wands* factors discussed below, undue experimentation is not required by one reasonably skilled in the art to practice the claimed methods of using an article having a nonfouling surface thereon.

The claimed subject matter relates to methods of using an article having a nonfouling surface thereon, said method comprising providing an article having a nonfouling surface thereon, said article comprising: *(i)* a substrate having a surface portion; *(ii)* a linking layer on said surface portion; and *(iii)* a polymer layer formed on said linking layer by the process of surface-initiated polymerization of monomeric units thereon, with each of said monomeric units comprising a vinyl monomer core group having at least one protein-resistant head group coupled thereto, to thereby form a brush molecule on said surface portion; said brush molecule comprising a stem formed from the polymerization of said monomer core groups, and a plurality of branches formed from said protein-resistant head group projecting from said stem, and wherein said brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2; and then contacting said article to a biological fluid, and where proteins in said fluid do not bind to said surface portion.

As the Examiner has acknowledged, the relative skill of those in the art is high. The Examiner has provided that the level of skill is that of a MD or PhD. Accordingly, Applicant respectfully submits that this factor weighs in favor of the Applicant.

However, the Examiner contends that the art is unpredictable and cites Flynn et al. (*Langmuir* 2003 19:10909-10915) as supporting this proposition. Applicant respectfully disagrees and submits that Flynn et al. is not directed to the claimed brush polymers, but rather to the formation of self-assembled monolayers (SAMs) of pre-formed polymers of undecanethiol ($C_{11}H_{23}SH$) and tri(ethylene glycol)-terminated undecanethiol ($HO(C_2H_4O)_3C_{11}H_{22}SH$) on gold surfaces. *See,* Flynn et al., Abstract and page 10910, column 2, first paragraph. Thus, the polymers described in Flynn et al. are not an adequate representation of the stability of the claimed brush polymers.

Some of the differences between the SAMs described in Flynn et al. and the claimed brush polymers are described in the specification. Specifically, the specification, like Flynn et al., provides that $(EG)_n$-SH SAMs have limited robustness and loss of cell resistance because they are a single molecular layer and because of the propensity of the thiolate to oxidize. Specification, paragraph [0005]. In contrast, the claimed brush polymers comprise a linking layer on the surface portion and a polymer layer formed on the linking layer by the process of surface-

initiated polymerization of monomeric units thereon, with each of said monomeric units comprising a vinyl monomer core group having at least one protein-resistant head group coupled thereto, to thereby form a brush molecule on said surface portion. Further, the specification provides that the architecture of $(EG)_n$-SH SAMs is considerably different than the architecture of the brush polymers. Specification, paragraph [0077]. Moreover, the claimed brush polymers have a tunable thickness in the 5-50 nm range, a high brush polymer density, and exhibit improved protein and cell resistance. This thickness is inaccessible to SAMs, such as those of Flynn et al., and can easily be synthesized by surface-initiated polymerization. Specification, paragraph [0067]. Thus, the polymers described in Flynn et al. and the claimed brush polymers are quite different from each other.

Even if the polymers described in Flynn et al. could be correlated to the stability of the claimed brush polymers, Flynn et al. describes a study done by Schoenfisch et al. that demonstrated improved device function and biocompatibility *in vivo* despite the long-term degradation of the monolayer. Flynn et al. states that "[t]his indicates that even a short period of stability for these methyl-terminated alkanethoils imparts useful long-term effect *in vivo*. Similar and perhaps more dramatic effects may be observed through device surface modification with oligo-(ethylene glycol)-terminated SAMs." Flynn et al., page 10915, col. 1, paragraph 1. Thus, based on the disclosure in Flynn et al. one skilled in the art would not be directed that instability of an alkanethiol self-assembled monolayer would destroy long term use. Accordingly, despite potential instability, as suggested by the Examiner, one skilled in the art could use the claimed methods without undue experimentation.

The Examiner also asserts that Applicant acknowledges and highlights the instability of alkanethiol self-assembled monolayers in their prior arguments. Applicant respectfully submits that the Examiner has taken Applicant's argument out of context. The Examiner is referring to Applicant's argument regarding Healy in response to the Examiner's prior argument that it would have been obvious based on the disclosure of Healy in combination with Chapman et al., Zhang et al., and Hawker et al. to implant (*in vivo* contacting) an orthopedic device made obvious by these references. Applicant reiterates that Healy is not directed to the claimed nonfouling surfaces, but rather to surfaces designed to promote specific cellular interactions, such as the binding of specific proteins. Moreover, Healy does not teach or suggest the claimed brush polymers and instead describes monolayers of self-assembled thiol-linked molecules. Accordingly, there are substantial differences between the claimed subject matter and the

disclosure of Healy. The portion in Healy describing the stability of self-assembled monolayers based on gold-thiol chemistry *in vivo* was directed to a study done relating to the absorption of proteins on SAMs, which were not brush polymers, to control cell behavior. Thus, the disclosure referred to in Healy is directed to a use contrary to the claimed subject matter and a polymer different from the claimed brush polymers. Accordingly, Applicant was not referring to the stability of the claimed brush polymers, but to the polymers described in Healy in response to the Examiner's argument regarding *in vivo* application.

Moreover, Applicant respectfully submits that the Examiner is misinterpreting the teachings of the cited references. The Examiner is focusing on the stability of self-assembled monolayers on gold based on their attachment to gold through a thiol group. While the references may describe thiol-linked self-assembled monolayers on gold this does not make the polymers comparable to one another. Applicant submits that the properties of polymer surface coatings are dependent on more than just their linkage to the substrate, and as described in the specification, also depend on properties such as surface density and thickness of the polymer. Applicant respectfully submits that none of the cited references have described the claimed brush polymers.

The Examiner alleges that the "specification provides no direction or guidance for how to practice the invention as claimed beyond the 26 days exemplified." Office Action, page 5. Applicant respectfully disagrees and submits that examples are provided in the specification showing the claimed method beyond 26 days. Specifically, the specification provides examples of $(EG)_n$ functionalized polymer brushes and describes that these brush polymers have a tunable thickness in the range of 5-20 nm and exhibited "no detectable adsorption of proteins and are cell resistant for up to a month." Specification, paragraph [0067]. Additionally, retention of cellular patterns was observed with poly(OEGMA) brush polymers for up to 30 days. Specification, paragraph [0076]. Accordingly, Applicant submits that there are working examples in the specification for how to practice the claimed method beyond 26 days. Thus, this factor weighs in favor of the Applicant.

Moreover, the specification provides guidance on how to practice the claimed method and how to provide the non-fouling properties of the claimed subject matter. Specifically, the specification provides that "[t]he contacting step may be carried out acutely or chronically: *e.g.*, for a period of at least one day, one week, one month, one year, etc., depending upon the particular article being utilized." Specification, paragraph [0064]. The specification describes

that low surface density of PEG chains limits their resistance to proteins and cells. In contrast, the claimed polymer brushes have a high brush polymer density and a tunable thickness in the 5-50 nm range that can be prepared by surface-initiated polymerization, which provides control over the chain length and surface density. Specification, paragraphs [0068] and [0077]. Furthermore, the specification teaches that protein resistance for the claimed polymer brushes is believed to be controlled by two primary structural features that contribute to the non-fouling properties of $(EG)_n$-SH SAMs, which are: terminal hydrophilicity of the head-group combined with the formation of a dense, but disordered $(EG)_n$ brush with significant penetration of water into the $(EG)_n$-SH SAMs. Specification, paragraph [0073]. The specification teaches at paragraph 41 that numerous initiators in addition to thiol-initiators can be used to carry out the present invention, and indeed other materials within the present invention have been readily made by such processes with other initiators, and which demonstrate the present invention. ***See, e.g.,*** **H. Ma. et al.,** ***Protein Resistant Polymer Coatings on Silicon Oxide by Surface-Initiated Atom Transfer Radical Polymerization,*** **Langmuir 22, 3751 (2006)(copy submitted concurrently herewith).** Thus, based on these teachings, one skilled in the art would be able to practice the claimed method beyond 26 days without undue experimentation. Thus, this factor weighs in favor of the Applicant.

Applicant respectfully asserts that the enablement requirement simply requires that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art. Moreover, Applicant submits that the enablement requirement is met if the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Given the discussion above, particularly the level of skill in the art, the examples, and teachings in the specification, Applicant respectfully submits that there is no reason to doubt that the enablement requirement is satisfied for the claimed invention. Therefore, Applicant respectfully requests that this rejection be withdrawn.

III. Rejections under 35 U.S.C. § 103(a)

A. Rejection in view of Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al.

Claims 2, 4, 11-14, 21, 28-31, 51, and 53 stand rejected as allegedly being obvious under 35 U.S.C. § 103(a) in view of Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al.

To narrow and simplify the issues, claims 28 and 51 have been amended herein to incorporate the subject matter of claim 17. Accordingly, independent claims 28 and 51 recite that the brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2. As the Examiner has acknowledged, none of the above cited references, alone or in combination, teach or suggest this claimed feature. Office Action, page 10. Accordingly, Applicant respectfully submits that this rejection is moot for at least this reason and requests its withdrawal.

B. Rejection in view of Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. and further in view of Allbritton et al. and Leckband et al.

Claims 17 and 28 stand rejected as allegedly being obvious under 35 U.S.C. § 103(a) in view of Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. and further in view of Allbritton et al. (U.S. Patent Publication No. 2005/023748) and Leckband et al.

As an initial matter, Applicant submits that the combination of Mittelman in view of Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. fails to teach or suggest the claimed method of using an article having a nonfouling surface thereon. Mittelman describes the adhesion of bacteria to biomaterials, but fails to teach or suggest an article having a nonfouling surface, yet alone a nonfouling surface comprising a brush polymer. Bialk et al. describes the grafting of polymers to solid surfaces by using immobilized methacrylates. The Examiner alleges that "[i]n light of the teachings of Bialk et al., it would have been obvious to one of ordinary skill in the art at the time of the invention to graft a polymer that resists bacterial adhesion from the surface of a joint replacement prior to its implantation." Office Action, page 9. However, Bialk et al. also fails to teach or suggest an article having a nonfouling surface thereon, yet alone a nonfouling surface comprising a brush polymer. As can be seen from Figure 1(a) of Bialk et al., the graft polymers of Bialk et al., which are grown by attachment, are quite different from a brush polymer. *See, e.g.*, Specification, Figure 1A. The disclosures of Zhang et al., Ejaz et al., and Chapman et al. fail to correct these deficiencies. Specifically, Applicant reiterates that Zhang et al. is not directed to polymer brushes as the Examiner alleges, but rather to freestanding films or films coated on (essentially painted on) a surface. Zhang et al. describes that PMB, a pre-formed polymer, was coated onto underlying matrices. *See*, p. 692, col. 1, first and second full paragraphs; col. 2, second paragraph; and Figure 1. Ejaz et al. is directed to the polymerization of glycopolymers. Finally, Chapman et al. is also not directed to the claimed brush polymers, but instead to self-assembled monolayers on gold to provide reactive groups, to which pre-formed

polymer is then attached, and then to which kosmotropes are in turn attached. Accordingly, none of the above cited references, alone or in any combination, teach or suggest the claimed article having a nonfouling surface thereon, wherein the article comprises a brush molecule.

Moreover, claim 28, has been amended herein to incorporate the subject matter of claim 17 and recites that the brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2. Given this amendment to claim 28, claim 17 has been cancelled herein. As the Examiner has acknowledged, the combination of Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. fails teach or suggest this claimed feature. Office Action, page 10. Thus, the subject matter of claim 28 is also nonobvious for the below mentioned reasons.

Specifically, the Examiner asserts that Allbritton et al. describes "grafting densities from approximately 5 $\mu g/cm^2$ (50 mg/m^2) to 60 $\mu g/cm^2$ (600 mg/m^2)." Office Action, page 10. Leckband et al. is cited by the Examiner as describing that "the graft density (polymer surface density) is a key parameter in controlling the degree of protein adsorption retardation." Office Action, pages 10-11. Thus, the Examiner contends that "it would have been well within the purview of one of ordinary skill in the art to optimize the grafting density of the polymers of Mittelman in view of Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. based upon the teachings of Allbritton et al." Office Action, page 11. However, Applicant respectfully disagrees.

As the Examiner notes, Allbritton et al. is directed to graft polymers. Applicant submits that not only are the graft polymers described in Allbritton et al. a different type of polymer than the claimed brush polymers, but that the graft polymers of Allbritton et al. are formed by a different method than the claimed method. Specifically, the graft polymers of Allbritton et al. are covalently attached to a polymer substrate surface by ultraviolet graft polymerization. *See*, Allbritton et al., Abstract. As shown in Figure 1 and described at paragraph [0048], the monomers described in Allbritton et al. are grafted onto the polymer backbone of the polymer substrate surface.

In contrast, the claimed method comprises an article having a nonfouling surface thereon, wherein the article comprises a substrate have a surface portion, a linking layer on said surface portion, and a polymer layer formed on said linking layer by the process of surface-initiated polymerization of monomeric units thereon, with each of said monomeric units comprising a vinyl monomer core group having at least one protein-resistant head group coupled thereto, to thereby form a brush molecule on said surface portion. As an initial matter, it is Applicant's

understanding that the elected species for the surface portion is a metal, which Allbritton et al. does not teach or suggest. In fact, Allbritton et al. teaches away from this species by their requirement of a polymer surface substrate. Moreover, Allbritton et al. does not teach or suggest a linking layer on the surface portion. Instead, Allbritton et al. describes covalent attachment of the monomers directly to the surface of the substrate. *See*, Allbritton et al., Figure 1 and paragraph [0018]. Furthermore, Allbritton et al. fails to teach or suggest a polymer layer formed on the linking layer, yet alone a polymer formed on the linking layer by the process of surface-initiated polymerization of monomeric units thereon, with each of said monomeric units comprising a vinyl monomer core group having at least one protein-resistant head group coupled thereto, to thereby form a brush molecule on said surface portion.

Thus, the graft polymers described in Allbritton et al. are not only different from the claimed brush polymers, but also formed by a different process. Accordingly, there is no teaching or suggestion that would have motivated one skilled in the art to combine the process of Allbritton et al. with the above cited references to achieve the claimed brush polymer density. Nor is there any reasonable expectation of success given these differences. Moreover, Leckband et al. fails to teach or suggest the claimed brush polymer density. Therefore, Applicant respectfully submits that claim 28 is nonobvious over the above cited references and requests withdrawal of this rejection.

Applicant further submits that the Examiner cites to different types of polymers proposed by different prior art references to allege that the claimed combination of features is obvious. However, Applicant respectfully disagrees and notes that the U.S. Supreme Court has provided that a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. *KSR International Co. v. Teleflex Inc., et al.* 550 U.S. 1, 12 (2007) at 14. The Examiner's reference to Zhang et al., Chapman et al., and Allbritton et al., for example, which are clearly directed to very different types of polymers to select isolated features to combine to render the claimed invention obvious, appears to fall into an improper (hindsight) analysis.

Applicant respectfully submits that the Examiner combines different references based on the teachings of the instant invention. Applicant respectfully reminds the Examiner that selective combination of references based only on the suggestions of the instant application is improper. "Hindsight is a tempting but forbidden zone." *Loctite Corp. v. Ultraseal Ltd.*, 228, USPQ 90, 98 (Fed. Cir. 1985). The standard of obviousness is not whether, in hindsight, it seems elementary

that someone would have combined the elements in the prior art to form the invention in question. *See W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 220 USPQ 303 (Fed. Cir. 1983).

Again, the fact that the invention employs known elements does not preclude patentability. It is the claimed combination of elements which is the proper basis for review. "Virtually all inventions are necessarily combinations of old elements." *Panduit* at 1575 (citing *Medtronic, Inc. v. Cardiac Pacemakers, Inc.*, 220 USPQ 97, 99-100 (Fed. Cir. 1983)). Indeed, the Federal Circuit has stated (in regard to an obviousness-type invalidity challenge to an issued patent) that "[t]he notion, therefore, that combination claims can be declared invalid merely upon finding similar elements in separate prior patents would necessarily destroy virtually all patents and cannot be the law under the statute, §103." *Panduit* at 1575.

C. Rejection in view of Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. and further in view of Guan et al.

Claims 8-9 and 28 stand rejected as allegedly being obvious under 35 U.S.C. § 103(a) in view of Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. and further in view of Guan et al. To narrow and simplify the issues, claim 28 has been amended herein to incorporate the subject matter of claim 17. Accordingly, independent claim 28 recites that the brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2. As the Examiner has acknowledged, Mittelman, Bialk et al., Zhang et al., Ejaz et al, and Chapman et al. fail to teach or suggest this claimed feature. Office Action, page 10. Guan et al. fails to correct this deficiency. Accordingly, Applicant respectfully submits that this rejection is moot for at least this reason and requests its withdrawal.

D. Rejection in view of Chapman et al. in view of Hawker et al., Zhang et al., Morgan, and Flynn et al.

Claims 2, 4-7, 9, 11-14, 16, 18-19, 21, 28-31, 51, and 53 stand rejected as allegedly being obvious under 35 U.S.C. § 103(a) in view of Chapman et al. in view of Hawker et al., Zhang et al., Morgan (U.S. Patent No. 6,325,628), and Flynn et al. To narrow and simplify the issues, claims 28 and 51 have been amended herein to incorporate the subject matter of claim 17. Accordingly, independent claims 28 and 51 recite that the brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2. As the Examiner has acknowledged, none of the above cited references, alone or in combination, teach or suggest this

claimed feature. Office Action, page 17. Accordingly, Applicant respectfully submits that this rejection is moot for at least this reason and requests its withdrawal.

E. Rejection in view of Chapman et al. in view of Hawker et al., Zhang et al., Morgan, and Flynn et al. and further in view of Allbritton et al. and Leckband et al.

Claims 17 and 28 stand rejected as allegedly being obvious under 35 U.S.C. § 103(a) in view of Chapman et al. in view of Hawker et al., Zhang et al., Morgan (U.S. Patent No. 6,325,628), and Flynn et al. and further in view of Allbritton et al. and Leckband et al.

As an initial matter, Applicant submits that the combination of Chapman et al. in view of Hawker et al., Zhang et al., Morgan, and Flynn et al. fails to teach or suggest the claimed method of using an article having a nonfouling surface thereon. As described above, Chapman et al. is not directed to the claimed brush polymers, but instead to self-assembled monolayers on gold to provide reactive groups, to which pre-formed polymer is then attached, and then to which kosmotropes are in turn attached. Morgan et al. is cited by the Examiner as describing an implant for *in vivo* contacting with blood, but also fails to teach or suggest the an article having a nonfouling surface thereon, yet alone a nonfouling surface comprising a brush polymer. The disclosures of Zhang et al., Hawker et al., and Flynn et al. fail to correct these deficiencies. Specifically, Applicant reiterates that Zhang et al. is not directed to polymer brushes as the Examiner alleges, but rather to freestanding films or films coated on (essentially painted on) a surface. Zhang et al. describes that PMB, a pre-formed polymer, was coated onto underlying matrices. *See*, p. 692, col. 1, first and second full paragraphs; col. 2, second paragraph; and Figure 1. Hawker et al. is directed to growing a film *in situ* on a surface, and does not suggest applying protein-resistance to a surface. Finally, Flynn et al., as described in more detail above, is directed polymers whose architecture is quite different from the claimed brush polymers. Accordingly, none of the above cited references, alone or in any combination, teach or suggest the claimed article having a nonfouling surface thereon, wherein the article comprises a brush molecule.

Moreover, claim 28, has been amended herein to incorporate the subject matter of claim 17 and recites that the brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2. Given this amendment to claim 28, claim 17 has been cancelled herein. As the Examiner has acknowledged, the combination of Chapman et al., Hawker et al., Zhang et al., Morgan, and Flynn et al. fails teach or suggest this claimed feature. Office Action,

page 17. Thus, the subject matter of claim 28 is also nonobvious for the below mentioned reasons.

Specifically, the Examiner asserts that Allbritton et al. describes "grafting densities from approximately 5 $\mu g/cm^2$ ($50mg/m^2$) to 60 $\mu g/cm^2$ ($600mg/m^2$)." Office Action, page 17. Leckband et al. is cited by the Examiner as describing that "the graft density (polymer surface density) is a key parameter in controlling the degree of protein adsorption retardation." Office Action, pages 17-18. Thus, the Examiner contends that "it would have been well within the purview of one of ordinary skill in the art to optimize the grafting density of the polymers of Chapman et al. in view of Hawker et al., Zhang et al., and Morgan based upon the teachings of Allbritton et al." Office Action, page 18. However, Applicant respectfully disagrees.

As described in more detail above, the graft polymers described in Allbritton et al. are not only a different type of polymer than the claimed brush polymers, but also are formed by a different method than the claimed method. Specifically, the graft polymers of Allbritton et al. are covalently attached to a polymer substrate surface by ultraviolet graft polymerization. *See*, Allbritton et al., Abstract. In contrast, the claimed method comprises an article having a nonfouling surface thereon, wherein the article comprises a substrate have a surface portion, a linking layer on said surface portion, and a polymer layer formed on said linking layer by the process of surface-initiated polymerization of monomeric units thereon, with each of said monomeric units comprising a vinyl monomer core group having at least one protein-resistant head group coupled thereto, to thereby form a brush molecule on said surface portion.

Accordingly, there is no teaching or suggestion that would have motivated one skilled in the art to combine the process of Allbritton et al. with the above cited references to achieve the claimed brush polymer density. Nor is there any reasonable expectation of success given these differences. Moreover, Leckband et al. fails to teach or suggest the claimed brush polymer density. Therefore, Applicant respectfully submits that claim 28 is nonobvious over the above cited references and requests withdrawal of this rejection.

Additionally, as described in more detail above, Applicant respectfully submits that the Examiner combines different references based on the teachings of the instant invention. Applicant respectfully reminds the Examiner that selective combination of references based only on the suggestions of the instant application is improper. "Hindsight is a tempting but forbidden zone." *Loctite Corp. v. Ultraseal Ltd.*, 228, USPQ 90, 98 (Fed. Cir. 1985). The standard of obviousness is not whether, in hindsight, it seems elementary that someone would have combined

the elements in the prior art to form the invention in question. *See W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 220 USPQ 303 (Fed. Cir. 1983).

Again, the fact that the invention employs known elements does not preclude patentability. It is the claimed combination of elements which is the proper basis for review. "Virtually all inventions are necessarily combinations of old elements." *Panduit* at 1575 (citing *Medtronic, Inc. v. Cardiac Pacemakers, Inc.*, 220 USPQ 97, 99-100 (Fed. Cir. 1983)). Indeed, the Federal Circuit has stated (in regard to an obviousness-type invalidity challenge to an issued patent) that "[t]he notion, therefore, that combination claims can be declared invalid merely upon finding similar elements in separate prior patents would necessarily destroy virtually all patents and cannot be the law under the statute, §103." *Panduit* at 1575.

F. Rejection in view of Chapman et al. in view of Hawker et al., Zhang et al., Morgan, and Flynn et al. and further in view of Guan et al.

Claims 8 and 28 stand rejected as allegedly being obvious under 35 U.S.C. § 103(a) in view of Chapman et al. in view of Hawker et al., Zhang et al., Morgan (U.S. Patent No. 6,325,628), and Flynn et al. and further in view of Guan et al. To narrow and simplify the issues, claims 28 has been amended herein to incorporate the subject matter of claim 17. Accordingly, independent claim 28 recites that the brush molecule is formed on said surface portion at a density from 40 to 100 milligrams per meter^2. As the Examiner has acknowledged, Mittelman, Bialk et al., Zhang et al., Ejaz et al, fail to teach or suggest this claimed feature. Office Action, page 17. Guan et al. fails to correct this deficiency. Accordingly, Applicant respectfully submits that this rejection is moot for at least this reason and requests its withdrawal.

IV. New claims 60-63

New claims 60 and 61 are directed to particular embodiments of the present invention and are added herein to complete the record. Support for new claims 60 and 61 can be found, at least, in the specification at paragraph [0064]. Applicant respectfully submits that new claims 60 and 61 are believed to be free of all the rejections cited above for pending claims 2-9, 11-31, and 51-59. Thus, entry and allowance of new claims 60 and 61 is respectfully requested.

Conclusion

It is respectfully submitted that this application is in condition for allowance, which action is respectfully requested. Should there be any remaining concerns, the Examiner is encouraged to contact the undersigned attorney by telephone to expedite the prosecution of this application.

Respectfully submitted,

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ENCLOSURE: H. Ma. et al., Langmuir 22, 3751 (2006)

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Protein-Resistant Polymer Coatings on Silicon Oxide by Surface-Initiated Atom Transfer Radical Polymerization

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The modification of silicon oxide with poly(ethylene glycol) to effectively eliminate protein adsorption has proven to be technically challenging. In this paper, we demonstrate that surface-initiated atom transfer radical polymerization (SI-ATRP) of oligo(ethylene glycol) methyl methacrylate (OEGMA) successfully produces polymer coatings on silicon oxide that have excellent protein resistance in a biological milieu. The level of serum adsorption on these coatings is below the detection limit of ellipsometry. We also demonstrate a new soft lithography method via which SI-ATRP is integrated with microcontact printing to create micropatterns of poly(OEGMA) on glass that can spatially direct the adsorption of proteins on the bare regions of the substrate. This ensemble of methods will be useful in screening biological interactions where nonspecific binding must be suppressed to discern low probability binding events from a complex mixture and to pattern anchorage-dependent cells on glass and silicon oxide.

Introduction

Glass and silicon oxide are widely used substrates for biosensors, clinical diagnostics, and cell culture[1] and as solid supports for the synthesis of peptides, carbohydrates, and DNA.[2] The modification of silicon oxide to modulate protein and cell interactions has proven to be challenging for a number of technical reasons. First, the formation of silane self-assembled monolayers (SAMs), the most common route to functionalize glass (and other metal oxides), is complicated by the sensitivity of most silanes to humidity and their related propensity to form polymeric multilayers.[3,4] Furthermore, as is typical to most "grafting to" approaches, the passivation of silicon oxide by grafting PEG to the surface using silane chemistry[5,6] does not provide a high surface density of PEG because of the excluded volume effect.[7] Consequently, grafted PEG coatings on glass decrease the adsorption of proteins, but do not eliminate their adsorption below the nominal limit of several nanograms per square centimeter. For example, Zhu and co-workers reported polymer grafts with a 92% surface density using low molecular weight PEG (weight average molecular weight, $M_W = 300$), but the surface density dropped to 35% for PEG of $M_W = 2000$.[8]

Several approaches have been taken to solve this problem, such as the sequential grafting of PEGs of different chain lengths and cloud-point grafting of PEG. In the former approach, the grafting of a longer chain length PEG (~5 kDa) is followed by that of a shorter chain length PEG (~2 kDa).[9] Although this underbrush grafting strategy results in a decrease in the adsorption of proteins, there is still detectable protein adsorption as measured by surface plasmon resonance (SPR). In the latter, "cloud-point" grafting approach, the excluded volume effect is reduced by grafting the polymer in a poor solvent, which improves the coating density over that obtained in a good solvent for PEG.[10] Furthermore, it is difficult to stamp silanes onto glass with the ease and reproducibility with which alkanethiols can be patterned by microcontact printing (μCP) and other soft lithography methods so that the patterning of PEG onto glass by soft lithography has only been marginally successful.[11,12]

Although much progress has been made in reducing protein adsorption on glass and other metal oxides, in our opinion, there remains a need for new strategies that: (1) can modify glass and other oxide surfaces to effectively eliminate the adsorption of proteins even from complex mixtures, such as serum and plasma; (2) can be carried out in situ in confined spaces with complex geometries, such as microfluidic bioanalytical devices;[13] (3) generate no residual polymer byproduct; and (4) can be patterned down to the microscale using simple and easy-to-use methods without the need for a clean room or complex equipment.

In this paper, we report on a set of methods that provide a solution to these problems. First, we demonstrate that surface-initiated atom transfer radical polymerization (SI-ATRP) of oligo(ethylene glycol) methyl methacrylate (OEGMA) (Figure 1A) can be successfully carried out on silicon oxide and glass under ambient conditions, and that these polymer coatings have excellent protein resistance in a biological milieu. We also demonstrate a new soft lithography method via which SI-ATRP is integrated with μCP to create micropatterns of poly(OEGMA) on glass that

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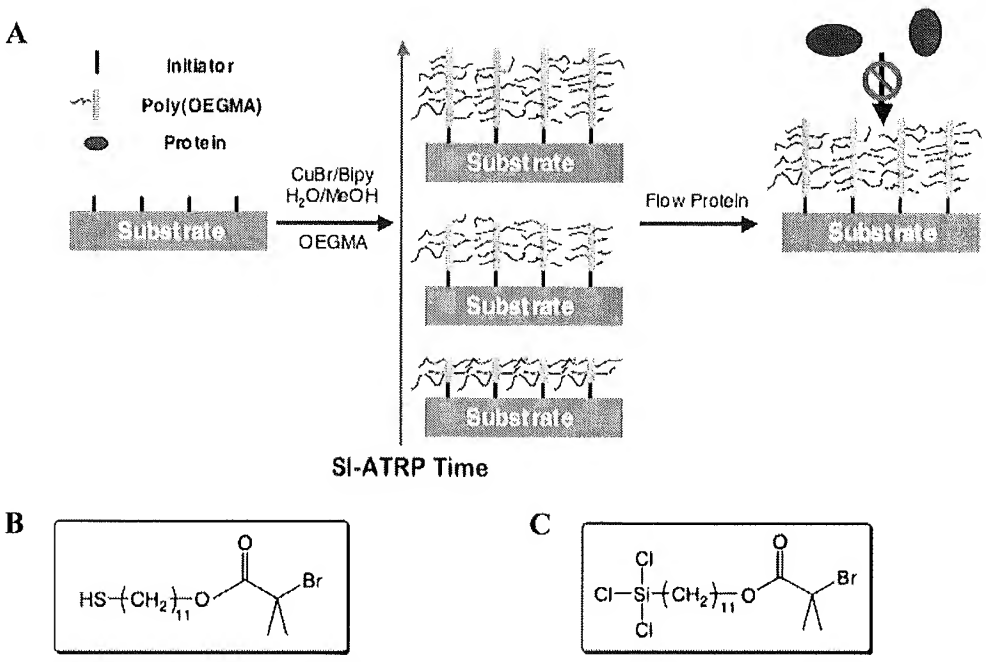

Figure 1. (A) Schematic illustration of SI-ATRP of OEGMA strategy to create a protein-resistant surface. Molecular structure of ATRP initiator; (B) thiol-terminated initiator, and (C) silane-terminated initiator, (11-(2-bromo-2-methyl)propionyloxy)undecyltrichloro silane (**1**).

can spatially direct the adsorption of proteins on the bare regions of the substrate.

Results and Discussions

Polymerization of OEGMA on Glass by SI-ATRP. Surface-initiated polymerization (SIP) first requires a suitable initiator to be immobilized onto a surface, followed by polymerization of a monomer of interest solely from the surface. In a previous study, we showed that SI-ATRP of OEGMA could be carried out on an alkanethiol SAM on gold that presented an ATRP initiator (Figure 1A).[14] We showed that SI-ATRP of OEGMA on gold allows in situ synthesis of a high surface density of oligo(ethylene glycol) chains at a surface, and that this coating conferred exceptional protein resistance and long-term stability in a biological milieu.[14] Subsequently, SI-ATRP of OEGMA has also been carried out by other investigators on glass and silicon oxide to improve their cell resistance[15] and as a coating for protein arrays,[16] though we note that neither of these studies directly examined their protein resistance or attempted to integrate SI-ATRP with μCP.

To carry out SI-ATRP of OEGMA on glass and silicon oxide, we chose a silane analogue of the initiator-terminated thiol with a spacer of 11 methylene units (Figure 1B and C) so as to retain the same initiator and its presentation on the surface as was used for gold.[14,17] The silane initiator, (11-(2-bromo-2-methyl)-propionyloxy)undecyltrichloro silane (**1**), was immobilized onto glass either by vapor deposition or by solution deposition. We chose vapor deposition of the silane to covalently present the ATRP initiator at the surface because the in situ synthesis of micropatterned poly(OEGMA) brushes on glass requires deposition of the silane without use of any organic solvents, so that solution deposition is incompatible with this micropatterning strategy. In other experiments that did not involve micropatterning, we also carried out SI-ATRP from silane layers that were deposited from solution because we hypothesized that it is likely to provide a higher surface density of the ATRP initiator than direct, vapor phase deposition that would likely impact the polymer surface density and thickness.

Table 1. Contact Angle and Ellipsometric Thickness Measurement (silicon wafer with ~180 Å thick silicon dioxide layer)

	Silane SAM		Poly(OEGMA) (Å)[d]	
	θ (°)[c]	ELM (Å)[d]	6 h SIP	18 h SIP
VD[a]	57.3 ± 0.6	12 ± 0.7	14 ± 0.6	95 ± 4.2
solution[b]	82.0 ± 0.4	23.9 ± 1.8	311.4 ± 4.8	1000.9 ± 16.1

[a] Silane SAM by vapor deposition (VD) method. [b] Silane SAM by solution dipping. [c] Contact angle. [d] Ellipsometry thickness measured in air−solid mode. Reported values were mean ± standard error, $n = 4$.

The vapor deposition of the initiator silane was carried out as follows: immediately after cleaning, a silicon wafer with a ~180 Å thick SiO_2 layer (oxide layer thickness measured by ellipsometry in air) was transferred to a vacuum desiccator in a glovebag that had been purged with nitrogen gas. The surface was modified by exposure to **1** under reduced pressure (~30 mmHg) for up to ~10 h to maximize the surface density of silane. The thickness of the silane layer was ~12 Å as measured by ellipsometry, and the sessile water contact angle was ~57°. Both the increase in thickness and increase in water contact angle compared to that of the SiO_2 substrate (<10°) indicated the modification of the silica substrate by the initiator silane (Table 1). In other experiments, the silane initiator layer was also prepared via solution deposition; substrates were incubated in a solution of **1** in toluene for ~10 min. The thickness of the silane layer was ~24 Å as measured by ellipsometry, and the sessile water contact angle was ~82° (Table 1).

SI-ATRP of OEGMA was then carried out by immersing the silane-modified substrate in a solution of the OEGMA monomer and CuBr/bipyridine as the catalyst system.[14,18] The thickness of the poly(OEGMA) film synthesized in situ by SI-ATRP from

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a surface modified by vapor deposition of the silane was ~14 and ~95 Å for ~6 and ~18 h polymerization time, respectively. Both poly(OEGMA) films had the same contact angle of 46 ± 1.0°. In contrast, using chips coated with a silane SAM of **1** prepared by solution deposition, poly(OEGMA) films with a significantly greater thickness of ~100 nm were synthesized by SI-ATRP for a polymerization time of ~18 h (Table 1).

We believe that these results can be explained, with some degree of approximation, by the increased surface density of the initiator presented by the solution-deposited silane SAM compared to vapor deposition. Compared with a value of ~18 Å for the analogous initiator thiol SAM on gold that is presumably densely packed due to self-assembly, a thickness of ~12 Å of a silane (**1**) SAM on glass by vapor deposition indicates incomplete monolayer formation while a thickness of ~24 Å obtained by solution deposition of **1** suggests the formation of a multilayer network of the silane on glass. We hypothesize that the silane multilayer presents a greater density of ATRP initiators with potentially greater accessibility on the surface compared to the submonolayer coverage of the vapor-deposited silane and results in a greater surface density of polymer chains rather than a polymer brush with a longer chain length. Because ellipsometry of the dry poly(OEGMA) film measures an effective optical thickness, we believe that the greater ellipsometric thickness for films grown from solution deposition of **1** compared to vapor deposition is reflective of the increased polymer surface density rather than chain length. We note that this scenario is consistent with previous results on poly(OEGMA) coatings that were synthesized from a mixed SAM on gold.[14,17]

The formation of a SAM of **1** and the resulting poly(OEGMA) film were further confirmed by XPS. The vapor phase deposition and solution deposition of the silane **1** introduced unique Br peaks with an atomic percentage (atom %) of ~1.1 and ~1.7 atom %, respectively, as quantified from their survey scan spectra (Figure 2A and Table 2). The C/O ratios for the poly(OEGMA) films of 10−100 nm thickness (greater than the sampling depth of XPS) varied between 1.9 and 2.6, which are in the range of 2.1 for stoichiometry of poly(OEGMA).

The growth of poly(OEGMA) on the surface had a significant effect on the intensity of the Si_{2s} peak, which decreased from 29.1% for the silicon oxide surface to 14.8% for a ~2 nm film, to 4.3% for a medium film, and was ≤2% for a ~100 nm film. The fact that the level of Si was highly variable, ranging from undetectable to a few atomic percent, suggests that it is caused by low molecular weight silicone oils that are highly surface active and are known to be ubiquitous surface contaminants. TOF−SIMS of these samples will help elucidate the probable cause of the Si signal, as PDMS has a characteristic mass spectral signature that is distinct from atomic silicon. This low and variable level of Si did not compromise the functional performance of the poly(OEGMA) coatings as they exhibited undetectable levels of protein adsorption in all cases.

High-resolution XPS of the C_{1s} photoemission envelope was carried out to determine the molecular composition of the poly(OEGMA) brushes as a function of film thickness. The high-resolution C_{1s} peak of the poly(OEGMA) brushes was fit with three unique carbon moieties (Figure 2B): CH_x (285.0 eV), C−O−R (286.7 eV), and COOR (289.1 eV).[19] SI-ATRP of OEGMA on glass clearly results in a significant increase in the ether component of the peak, consistent with the introduction of

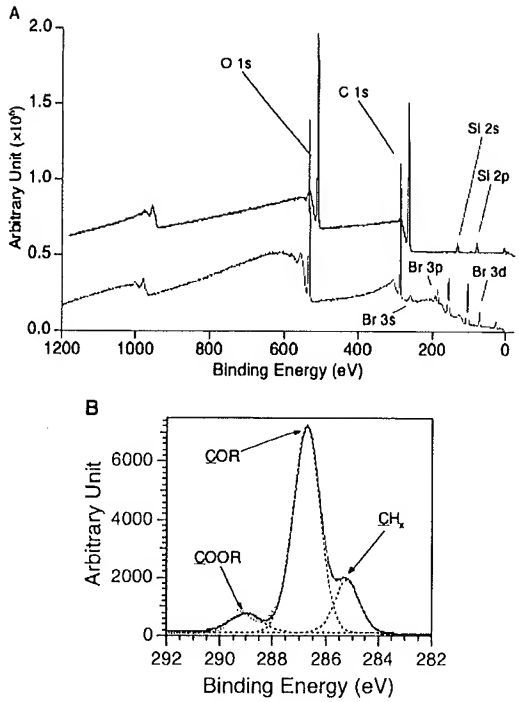

Figure 2. XPS. (A) Survey scan SAM of **1** prepared by solution deposition (gray curve) and survey scan of a ~1000 Å poly(OEGMA)-coated silicon wafer (black curve). The black curve was shifted in the *X* and *Y* direction for clarity. (B) High-resolution C_{1s} spectrum of poly(OEGMA) (thick film, ~1000 Å). Spectrum was fitted with three components: CH_X (~285 eV), COR (~286.7 eV), and COOR (~289 eV). Dashed gray lines are individual components, and the continuous black line is the overall envelope.

Table 2. Atomic Concentrations (atom %) of Initiator Silane SAM and Poly(OEGMA) Coatings on Silicon

	Elements[a]			
	C	O	Si	Br
silicon[b]	15.0	55.9	29.1	
VD SAM[c]	30.6	43.2	25.2	1.1
SAM[d]	54.3	27.0	17.0	1.7
thin coating[e]	41.9	43.3	14.8	
medium coating[e]	68.5	27.2	4.3	
thick coating[f]	70.4	27.2	2.4	

[a] The values were calculated from survey scan spectra: O_{1s} (~530 eV); C_{1s} (~285 eV); Si_{2s} (~153 eV); Br_{3d} (~69 eV). All peaks were referenced to Si_{2s} at ~153 eV. [b] The silicon wafer has an oxidized layer with thickness of ~180 Å [c] The layer of initiator silane was formed by ~10 h vapor deposition. [d] The layer of initiator silane was formed by solution deposition. [e] The thickness of poly(OEGMA) was ~15 and ~95 Å for thin and medium coatings, respectively. [f] Thickness of poly(OEGMA) was ~1000 Å for thick film of poly(OEGMA).

oligo(ethylene glycol) groups at the surface. From the deconvoluted C_{1s} spectrum of the ~100 nm thick poly(OEGMA) brush (Figure 2B), the ratio of components CH_x:C−O−R:COOR is 3:14:1.5 and is reasonably close to the 3:19:1 stoichiometry of the film assuming that $n = 9$ for the number of ethylene glycol repeats in OEGMA.[20]

Protein Resistance. The protein resistance of the poly(OEGMA) brushes was tested by the adsorption of fibronectin (Fn), bovine serum albumin (BSA), lysozyme (Ly) (all proteins at 1 mg mL^{-1} in PBS, pH = 7.4), and undiluted fetal bovine serum (FBS). Poly(OEGMA)-modified and the control (initiator-

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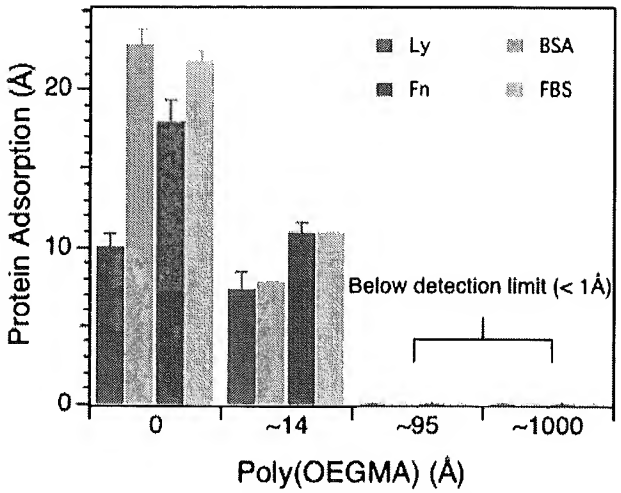

Figure 3. Protein adsorption onto the poly(OEGMA) brush on silicon oxide and initiator silane modified silicon oxide (control) measured by ellipsometry. The *x*-axis is the thickness of poly(OEGMA) coatings (or initiator silane as control, labeled as a film with 0 Å thickness), and the *y*-axis is the thickness of the adsorbed protein. Legend: Ly (lysozyme), Fn (fibronectin), BSA (bovine serum albumin), FBS (fetal bovine serum).

modified) silicon wafers were immersed into a protein solution for 1 h, rinsed with buffer, dried under a nitrogen stream, and the thickness of the adsorbed protein layer was measured by ellipsometry. The thickness of the protein layer as a function of the poly(OEGMA) film thickness is shown in Figure 3. The thickness of the adsorbed protein on the control surface varied depending upon the protein, ranging from ~10 (Ly) to ~25 Å for the other proteins and serum. In contrast, the poly(OEGMA) brush with ~14 Å thickness showed significantly less protein adsorption of all proteins and of serum. Increasing the thickness of the poly(OEGMA) brush to ~95 Å and above (~312 and ~1000 Å) eliminated the adsorption of all proteins and most notably that of serum to below the 1 Å detection limit of ellipsometry. These results on silicon are consistent with previous results on gold[14] that a dense and thick poly(OEGMA) brush on a surface completely resists protein adsorption from solution.

Protein Patterning by Soft Lithography. We also developed a new patterning technique by combining μCP,[21] vapor deposition of a silane,[22] and SI-ATRP[23] to create patterns of the poly(OEGMA) brush on the surface. Although this method uses μCP, it was designed to circumvent the need to directly μCP the silane on glass, which has proven to be difficult in previous attempts to use μCP to pattern silanes.[11,12] In this method, first a random copolymer of poly(methyl methacrylate-*co*-methoxy-terminated oligo(ethylene glycol) methacrylate) (referred to as poly(MMA-*co*-MeOEGMA)) was patterned by μCP on glass or silicon oxide wafers (Figure 4). The micropatterned poly(MMA-*co*-MeOEGMA) film functions as a mask for the subsequent vapor deposition of the initiator silane. After μCP, the patterned substrate was immediately transferred to a vacuum desiccator in a glovebag filled with nitrogen, and the silane was vapor deposited for ~10 h (Figure 4B) under reduced pressure. After vapor deposition of the silane, the poly(MMA-*co*-MeOEGMA) mask was removed by swabbing off the substrate in an ethanol/water mixture (80/20, v/v), and SI-ATRP of OEGMA was allowed to proceed as previously described (Figure 4C). Finally, the micropatterned surface was incubated in a solution of fibronectin, which led to the formation of micropatterns of fibronectin, due to adsorption of the protein only in regions that were not covered by the protein-resistant poly(OEGMA) brush (Figure 4D).

We note that any polymer can be used as the mask in the first step as long as it has the following attributes: (1) it must be amenable to being printed on a surface by μCP; (2) it should not chemically react with or be dissolved by the silane; and (3) it should be removed (preferably dissolved), without leaving any residue, in a solvent that has no effect upon the silane monolayer that is vapor deposited onto the surface. The poly(MMA-*co*-MeOEGMA) copolymer that we used has all of these attributes in that it can be easily patterned with high resolution by μCP on many different substrates;[20,24] it does not dissolve or react upon contact with the silane vapor; and finally, it is easily removed by an ethanol/water mixture. We have previously reported upon the use of a similar polymer for cell patterning,[20,24] and the polymer used here was identical to that reported previously except that all the OEG chains in the polymer used here were end-capped with methoxy groups to avoid possible reaction with **1**.

The different stages in the patterning of poly(OEGMA) brushes by this method are visualized in Figure 5. Figure 5A shows a phase contrast light microscopy image of 40 μm squares printed with the comb polymer with a pitch of 40 μm. Because poly(MMA-*co*-MeOEGMA) is protein resistant, we exploited this feature to visualize the printed polymer by incubation with biotinylated-Fn followed by Alexa488-labeled streptavidin (Figure 5B). The large difference in contrast ($I_{background}/I_{pattern}$ = 5.3)[20] between the background regions and the squares containing the printed polymer (baseline fluorescence) clearly shows the fidelity with which the polymer can be patterned on the surface. After deposition of a SAM of **1**, removal of the polymer, and SI-ATRP of poly(OEGMA) for ~18 h, incubation of the surface with biotinylated-Fn followed by Alexa488-labeled streptavidin resulted in a fluorescent pattern (Figure 5C) that is the inverse of the mask (Figure 5B) because the biotinylated-Fn only adsorbed to the regions that were not covered by the poly(OEGMA) brush that was synthesized in situ by SI-ATRP. The contrast ($I_{pattern}/I_{background}$) of 12 clearly shows the high S/N of Fn that is important to spatially direct the attachment of cells to patterned domains of the cell-adhesive protein.

There are several attractive features of this methodology. First, the use of SI-ATRP allows the thickness of the polymer brush to be controlled from several nanometers to ~10 nm, a range of thickness that is not easily accessed by other coating methodologies. In principle, by controlling the density of initiator sites at the surface,[25] this methodology should allow independent control of chain length and brush density, as we have demonstrated for the analogous system of alkanethiols on gold.[14,17] Second, we have demonstrated a method to micropattern these polymer brushes that uses μCP, which is attractive because of its simplicity, but circumvents the direct μCP of silanes, which is difficult. We also note that the methodology presented here is also applicable to other metal oxides that can be covalently modified with the initiator silane. In a similar approach, Fan et al. recently reported a bifunctional initiator that contains a catechol moiety to anchor the molecule to Ti or stainless steel by chemisorption and a terminal alkyl bromine that acts as an initiator for SI-ATRP. Using this approach, Fan et al. demonstrated the polymerization

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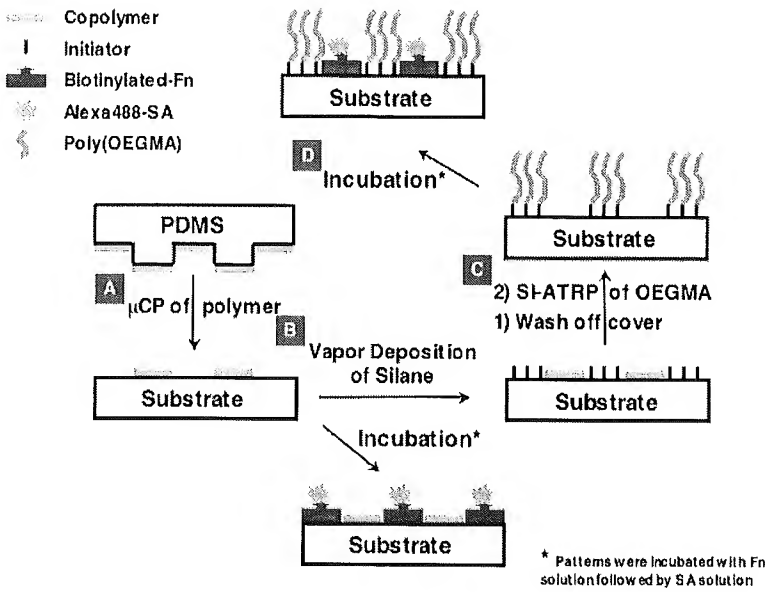

Figure 4. Pattern scheme. (A) Cleaned glass slides were first printed with comb polymer patterns as a mask and (B) subsequently coated with an initiator silane monolayer on the unpatterned area by vapor deposition of **1** in a desiccator. (C) After removal of the comb polymer mask, poly(OEGMA) patterns were formed by SI-ATRP of OEGMA from the ATRP initiator presented by the patterned SAM of **1**. (D) Patterned surfaces were incubated with biotinylated fibronectin followed by Alexa488-labeled streptavidin (Alexa488-SA).

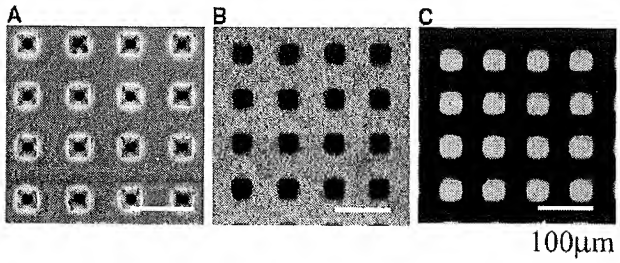

Figure 5. Pattern images. (A) A phase contrast light microscopy image of a comb polymer pattern fabricated by μCP. The raised area is the comb polymer, and the recessed area is the glass surface. (B) A confocal fluorescence image of the comb polymer pattern (dark, raised area containing the polymer) after incubation with biotinylated-Fn followed by Alexa488-labeled streptavidin (green). (C) A confocal fluorescence image of the substrate after silane monolayer formation, removal of comb polymer pattern, SI-ATRP of OEGMA for 18 h, and visualized by incubation with biotinylated-Fn and then Alexa488-labeled SA (green).

of ~100 nm thick films of OEGMA from the surface of Ti and stainless steel.[26]

The methodology developed here is also complementary to the poly(L-lysine)-*graft*-poly(ethylene glycol) graft copolymer (PLL-*g*-PEG) developed by Hubbell et al. and Textor et al.[27,28] in that both polymers confer protein resistance to glass and other metal oxides and can be patterned. We note that the PLL-*g*-PEG system is, to our knowledge, one of the few examples in which a polymer that is "grafted to" a surface exhibits an undetectable level of protein adsorption on glass that is comparable to poly(OEGMA) that is "grafted from" the surface. SI-ATRP of OEGMA has the advantage of providing a stable covalent link to the surface and will presumably prove to be more robust especially under high ionic strength conditions. In contrast, PEG/PLL is easier to implement as a simple "dip-and-rinse" process that is likely to be attractive for applications in which ease of sample preparation is of paramount importance. Although both polymers can be patterned, μCP of the polymer mask using a PDMS stamp is considerably simpler than the photoresist lift-off techniques developed to pattern PLL-*g*-PEG, though we parenthetically note that direct μCP of PLL-*g*-PEG should be possible. A major difference between the two systems, however, is that, because ATRP is a *pseudo*-living polymerization, the methodology presented here is amenable to the in situ synthesis of more complex macromolecular architectures by sequential polymerization of different monomers[23] than is possible by a grafting to strategy, such as chemisorption of PLL-*g*-PEG. This feature of SI-ATRP, in which a second polymer block can be elaborated upon the first poly(OEGMA) block, provides a powerful synthetic strategy to perform new chemistry upon a nonfouling surface template that may be of interest in the generation of small molecule,[29] peptide,[30,31] and carbohydrate arrays,[32,33] using supported solid phase synthesis[2] for screening of biological interactions where nonspecific binding must be suppressed to discern low probability binding events from a complex mixture.

In conclusion, the results reported here are notable and significant because they provide an experimentally simple ensemble of techniques that enable a high surface density of polymer chains that present pendant oligo(ethylene glycol) moieties to be immobilized on glass and silicon oxide. Furthermore, we show that the high surface density of oligo(ethylene glycol) has an important *functional* consequence: it reduces the adsorption of a complex protein mixture—serum—to below the detection limit of ellipsometry. Finally, we also demonstrate the extension of this methodology to micron scale patterning of protein using a variant of μCP.

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Experimental Section

Synthesis of ATRP Initiator Silane. (11-(2-Bromo-2-methyl)-propionyloxy)undecyl trichlorosilane (**1**) was synthesized by use of a similar procedure reported in the literature.[23,34] ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.26−1.68 (m, 20H), 1.91 (s, 6H), 4.14 (t, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm) 22.22, 24.27, 25.75, 28.31, 28.98, 29.13, 29.28, 29.41, 29.44, 30.76, 31.79, 56.00, 66.14, 171.74.

Initiator Silane Modification of Glass and Silicon Oxide. Glass slides were immersed into a 1% RBS 35 detergent solution (Pierce, Rockford, IL) for 10 min, sonicated in the detergent for another 10 min, and then rinsed with deionized water. The slides were immersed in a 1:1 (v/v) mixture of methanol:HCl for 30 min and rinsed copiously with deionized water and used immediately for silanization. SiO_2 wafers were cleaned by immersion in a mixture of NH_4OH:H_2O_2:H_2O (1:1:5, v/v/v) for 10 min at ~80 °C, followed by thorough rinsing in deionized water.

For vapor phase deposition of **1** under N_2 atmosphere, 1 mL of **1** was placed at the bottom of the desiccator in a glass Petri dish, and the wafer was subsequently placed ~5 cm over the bottom of the dish, with the SiO_2 side facing the silane source. The desiccator was then transferred out of the glovebag and immediately pumped down to a pressure of ~30 mmHg. The vapor deposition of **1** was allowed to proceed for ~10 h. After vapor deposition, the glass slides were rinsed with hot, deionized water (~80 °C) thoroughly to remove any physisorbed silane.

For solution deposition, **1** was added to toluene to a final concentration of ~0.05% solution (weight/volume) under nitrogen flow. Next, cleaned glass slides were immersed into this solution in a sealed tube, and silanization was allowed to proceed for 10 min. The silanized glass slides were then removed from the silane solution, rinsed with THF and hot water (~80 °C), and dried under a stream of N_2 gas.

SI-ATRP of Poly(OEGMA). SI-ATRP was carried out as reported previously.[14,17,18] Briefly, substrates were placed in a 100 mL flask under N_2. Next, CuBr (143 mg, 1.0 mmol), bipyridine (312 mg, 2.0 mmol), deionized water (degassed, 3 mL), methanol (12 mL), and OEGMA (8 g, 16.7 mmol) were mixed in a 50 mL flask, and the dark red solution was bubbled with N_2 gas for 30 min. The polymerization was initiated by adding the mixture to the flask and was continued for a specified time under nitrogen purge. The samples were pulled out of the solution to stop the polymerization, rinsed with methanol, and dried under a stream of N_2 gas.

Patterning of Poly(OEGMA) Brushes. A random copolymer of methyl methacrylate and methoxy-terminated oligo(ethylene glycol) methacrylate (poly(MMA-*co*-MeOEGMA)) was synthesized, as reported previously.[20,35] The polymer was microcontact printed on glass or silicon wafers, as reported previously.[20] Briefly, a solution of poly(MMA-*co*-MeOEGMA) (150 mg mL^{-1} in an ethanol:water mixture (80/20, v/v)) was spin coated onto a air plasma-treated PDMS stamp, and the polymer was transferred to the substrate by conformal contact. A SAM of **1** was then vapor deposited on the sample, as described in the previous section. The poly(MMA-*co*-MeOEGMA) pattern was then removed by gently swabbing the substrate with a Q-tip that was wet with an 80/20 (v/v) ethanol/water mixture.

Ellipsometry. Film thickness was measured in air on a M-88 spectroscopic ellipsometer (J. A. Woollam Co., Inc.) at angles of 65, 70, and 75° and wavelengths from 400 to 800 nm. A Cauchy layer model provided with the instrument was used for all organic films, and the ellipsometric data were fitted for thickness of SAMs and poly(OEGMA) film with fixed (A_n, B_n) values of (1.45, 0.01) and (1.46, 0.01), respectively. The data from three independent samples were averaged and reported as mean ± standard error.

X-ray Photoelectron Spectroscopy. XPS studies were performed on an AXIS Ultra electron spectrometer (Kratos Analytical, NY). Monochromatic Al Kα X-rays (1486.7 eV) were employed. Operational conditions for the X-ray source were 400 μm nominal X-ray spot size (fwhm) operating at 15 kV, 8.9 mA for both survey scan and high resolution core-level spectra. Survey spectra, from 0 to 1200 eV binding energy (BE), were recorded at a 100 eV pass energy with an energy step of 1.0 eV, a dwell time of 100 ms, for one scan. The operating pressure of the spectrometer was typically ~10^{-9} mbar. All peaks were referenced to Si_{2s} at ~153 eV for survey scan. All data were analyzed using the custom software provided by Casa XPS.

Contact Angle. The sessile water contact angles were measured on a Ráme-Hart goniometer (100-00, Mountain Lakes, NJ) using deionized water as the probe liquid. Substrates were rinsed with methanol and deionized water and dried under a stream of nitrogen before measurement. The contact angle (and ellipsometric thickness) for each sample was independently measured at three different locations. The data from three independent samples were average and reported as mean ± standard error.

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